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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07B 61/00, C07D 231/12, 261/08, 403/04

A1

(11) International Publication Number: **WO 97/19039**

(43) International Publication Date: 29 May 1997 (29.05.97)

(21) International Application Number: PCT/EP96/04808

(22) International Filing Date: 5 November 1996 (05.11.96)

(30) Priority Data:
95810717.9 17 November 1995 (17.11.95) EP
(34) Countries for which the regional or international application was filed: DE et al.

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(81) Designated States: AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: SOLID PHASE SYNTHESIS OF HETEROCYCLIC COMPOUNDS AND COMBINATORIAL COMPOUND LIBRARY

(57) Abstract

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SOLID PHASE SYNTHESIS OF HETEROCYCLIC COMPOUNDS AND COMBINATORIAL COMPOUND LIBRARY

The synthesis of combinatorial compound libraries is rapidly taking on the role of a powerful component within modern lead finding processes that aim at the identification of compounds with novel target activities of interest. In the drug discovery context, the ability to synthesize small organic molecules with high yield on a solid support has a definite strategic relevance. It facilitates the preparation of compound arrays in multiple parallel syntheses and enables the application of combinatorial methods for the synthesis of large libraries suitable for systematic evaluations in biochemical or biological test systems. In view of the expected biostability and bioavailability, small organics (e.g. heterocycles) rather than chain-like biooligomers are more attractive leads for subsequent medicinal chemistry efforts.

Here we report a scope and limitation study on a reaction sequence on solid phase, suitable for the generation of molecular diversity on small heterocycles of the pyrazole and isoxazole type. For each reaction, suitable conditions on solid phase were worked out and a variety of reactive agents (building blocks) was utilized in an effort to grasp the system's breadth of applicability. The inventive reaction sequence can be applied, for example, to exploit by the combinatorial approaches of the *split and mix* concept. Surprisingly, using the inventive method a new facile way for the synthesis of combinatorial compound libraries consisting of modified heterocyclic rings in high yields and purity is provided. These combinatorial compound libraries serve as valuable reservoirs for the screening for pharmaceutically active compounds.

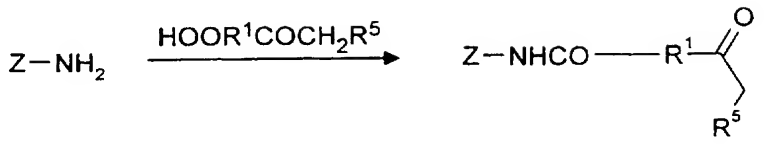
Detailed description of the invention

The current invention concerns a solid phase synthesis of a heterocyclic ring, characterized in that it comprises the following steps:

a) a solid carrier having reactive surface groups is loaded directely or via a spacer group with a compound bearing an acetyl function,
b) said acetyl function is modified using the Claisen condensation,
c) optional the reaction product of step b) is modified with an α-alkylation step, and
d) the heterocyclic ring is closed using a compound comprising two nucleophiles, wherein at least one of said nucleophiles is NH_2.

In a preferred embodiment of the invention

step a) is of formula 1



(1)

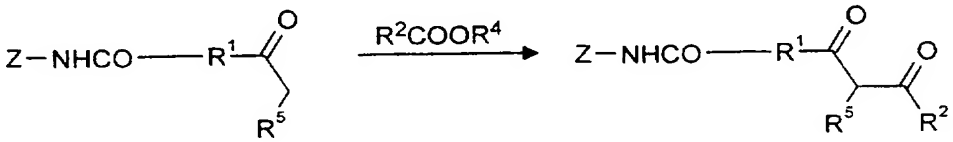
wherein

Z is a solid carrier;

R^1 is a substituted or unsubstituted conjugated system that has no acidic hydrogen atoms; and

R^5 is hydrogen, C_1-C_6alkyl, C_1-C_6alkylaryl, C_1-C_6alkoxy-C_1-C_6alkyl, C_1-C_6alkoxycarbonyl-C_1-C_6alkyl; each of which may be substituted or unsubstituted;

step b) is of formula 2



(2)

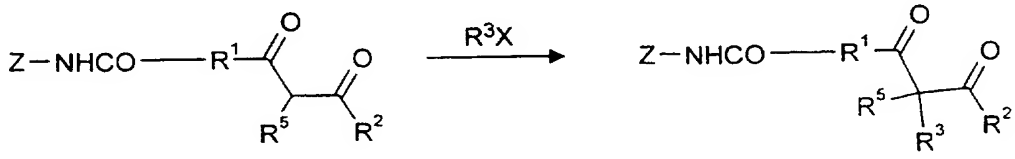
wherein

Z, R^1 and R^5 are defined as above;

R^2 is substituted or unsubstituted C_1-C_6alkyl, or a substituted or unsubstituted aromatic or aliphatic ring; and

R^4 is C_1-C_6alkyl, preferred is methyl and ethyl;

the optional step c) is of formula 3



(3)

wherein

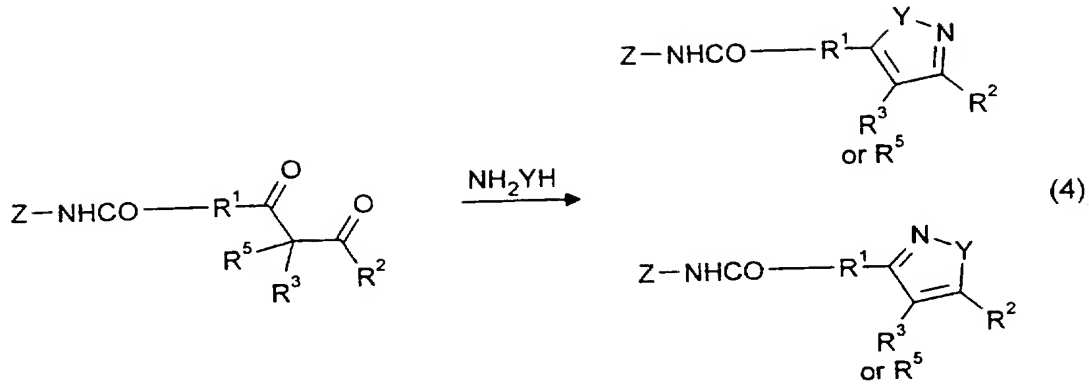
Z, R^1, R^2 and R^4 are defined as above;

R^5 is hydrogen

X is a halogen, preferred is I, Br or Cl; and

R^3 is C_1-C_6alkyl, C_1-C_6alkenyl, C_1-C_6alkylcarbonyl-C_1-C_6alkylene, arylcarbonyl-C_1-C_6alkylene, or a substituted or unsubstituted aromatic or aliphatic ring; and

step d) is of formula 4



(4)

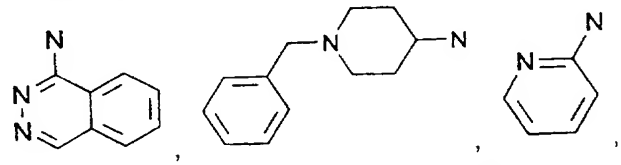
wherein

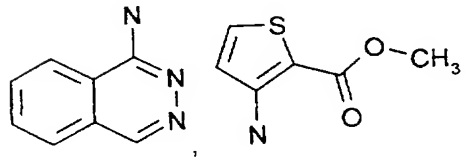
Z, X, R^1, R^2, R^3, R^4 and R^5 are defined as above;

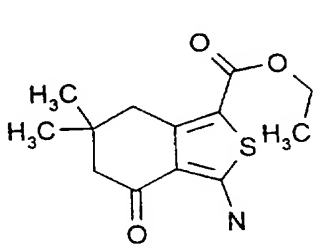
Y is a nucleophilic center like NH, NC_1-C_4alkyl, Naryl and O; preferred are O, NH, NC_1-C_4alkyl, NC_1-C_4alkyl aryl, Naryl unsubstituted or substituted with up to four Br, Cl, I, F, C_1-C_4alkyl, NO_2, SO_2C_1-C_4alkyl, OC_1-C_4alkyl, carboxy or carbonyl groups; suitable aryl groups are for example thienylene, thiantrenylene, furylene, phenoxanthiinylene, isobenzofuranylene, pyrazolylene, isothiazolylene, isoxazolylene, pyridinylene, pyrazinylene, pyrimidylene, indolizinylene, indazolylene, isoquinolylene, quinolylene, phthalazinylene, naphthyridinylene, quinoxalinylene, quinazolylene, cinnolinylene, phenylene, naphthylene;

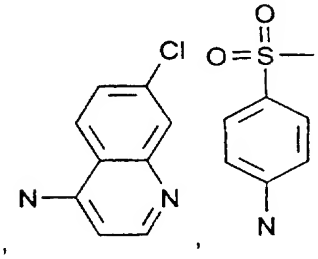
especially preferred are NH, O, NCH_3, NC_6H_5, $NCH_2C_6H_5$, $NCH_2COOC_2H_5$, NC_6H_4Cl, $NC_6H_3Cl_2$, NC_6H_4F, $NC_6H_3F_2$, NC_6HF_4, NC_6H_4Br, $NC_6H_4CH_3$, $NC_6H_3(CH_3)_2$, NC_2H_4OH, $NC_6H_4OCH_3$, $NC_6H_3Cl_2$, NC_6H_4Br, $NC_6H_4CF_3$, NC_6H_3ClF, NC_6H_{10}, NC_6H_4COOH,

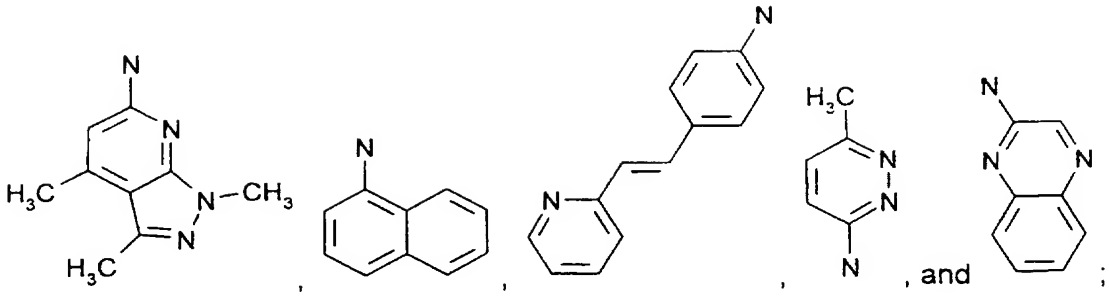
$NC_6H_3(NO_2)_2$, $NC_6H_4NO_2$, NCF_3, $NC(CH_3)_3$,









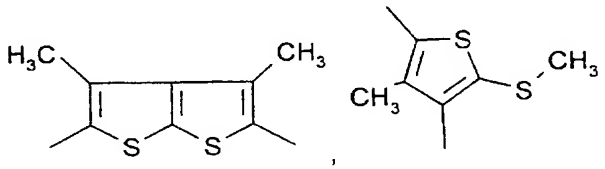


with the proviso that either R^3 or R^5 is hydrogen.

In a preferred embodiment of the invention the solid carrier Z is a resin. Usually Z is a particle that is insoluble in the reaction media and to which the ligand can be bound in sufficient amount by means of reactive groups at the surface of the this particle.

The binding of ligand and tag is effected, e.g. by amino, carboxyl, hydroxyl or halogen groups. These reactive groups are usually already constituents of the solid carrier, but they can also be applied or modified subsequently. The solid carrier customarily employed in solid-phase synthesis can be used, for example those used in Merrifield peptide synthesis. They consist largely of a polystyrene molecule that is crosslinked by copolymerization with divinyl benzene. The molecules are additionally derivatised to attach the reactants in the solid-phase synthesis.

In a more preferred embodiment of the invention R^1 is ethinylene, thienylene, thiantrenylene, furylene, phenoxanthiinylene, isobenzofuranylene, pyrazolylene, isothiazolylene, isoxazolylene, pyridinylene, pyrazinylene, pyrimidylene, indolizinylene, indazolylene, isoquinolylene, quinolylene, phthalazinylene, naphthyridinylene, quinoxalinylene, quinazolylene, cinnolinylene, phenylene, naphthylene,



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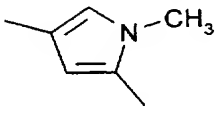
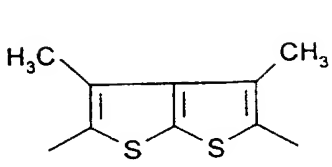
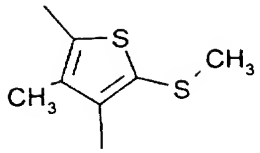


; wherein these conjugated systems are unsubstituted or substituted by groups that have no acidic hydrogens. Suitable groups are, for example, Cl, Br, I, CN, phenyl and pyridyl-groups. Especially preferred R^1 are benzene,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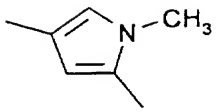
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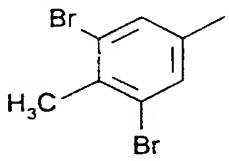
A preferred R^2 is, for example ethinyl, thienyl, thiantrenyl, furyl, phenoxanthiinyl, isobenzofuranyl, pyrazolyl, isothiazolyl, isoxazolyl, pyridinyl, pyrazinyl, pyrimidyl, indolizinyl, indazolyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolyl, cinnolinyl, phenyl and naphthyl; wherein these conjugated systems are unsubstituted or substituted by groups that have no acidic hydrogens. Especially preferred is phenyl, 4-$CH_3OC_6H_4$, 4-$ClC_6H_3(2Cl)$, 4-$CH_3OOCC_6H_4$, 4-NCC_6H_4, furyl, pyrrolyl, thienyl, pyridyl, methyl pyrridyl, pyrazinyl, $C_6H_5COOCH_3$, C_6F_5, $C_6H_4C(CH_3)_3$, $C_6H_4OCF_3$, $C_6H_4OCH_2C_6H_5$, $C_6H_4O,(CH_2)_{15}CH_3$, $C_6H_3(CF_3)_2$, $C_6H_4O(CH_2)_3CH_3$, C_6H_4Cl, C_6H_4CN, naphthyl, $C_6H_4N(CH_3)_2$, $C_6H_4C_6H_5$, $C_6H_4OCH_3$, $C_6H_4C_6H_4COOCH_3$, $C_6H_2(OCH_3)_3$, $C_6H_3Cl_2$,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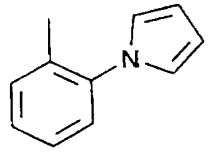
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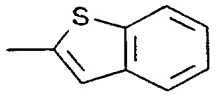
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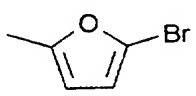
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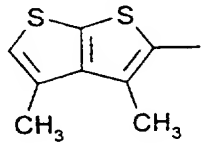
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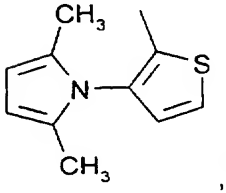
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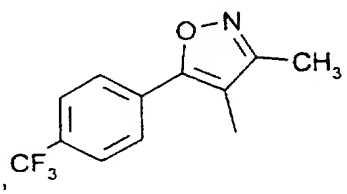
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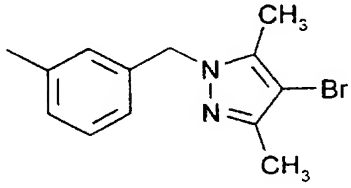
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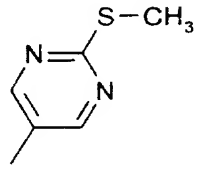
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A preferred R^3 is, for example, hydrogen, C_1-C_6alkyl, C_1-C_4alkoxy-C_1-C_4alkyl, C_1-C_6alkenyl, C_1-C_4alkoxy-aryl, C_1-C_5alkanoyloxy-C_1-C_6alkyl, C_1-C_6alkoxycarbonyl-C_1-C_5alkyl. Especially preferred is hydrogen, ethyl, $NCCH_2$, $CH_3CH_2OOCCH_2$, $C_6H_5COCH_2$ and CH_2=$CHCH_2$.

The inventive solid phase synthesis can be used for the generation of combinatorial compound libraries, e.g., in a the split and mix concept (Furka *et al.*, Abstr. 14th Int. Congr. Biochem., Prague (1988), **5**, 47; Furka *et al.*, Int. J. Peptide Protein Res. (1991), **37**, 487).

For example, in the first step the solid support is loaded with the R^1 component bearing the acetyl function (see table 1). Its carbonyl group is activated by standard methods and anchored to the acid labile Rink amide linker on polystyrene (Rink, Tetrahedron Lett. (1987), **28**, 3787). We observe quantitative transformations within 1 hour, unless ortho substituted

bifunctional derivatives like o-acetophenone and acetylphthalanilidic acid are used, which undergo ring closure side reactions (Nishio *et al.*, Heterocycl. Chem. (1995), **32**, 883).

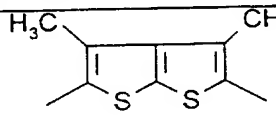
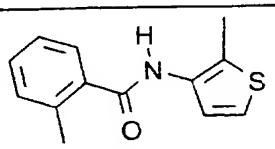
For the Claisen condensation, optimization of the reaction protocol with the prototypic aromatic ester ethyl benzoate identified conditions, which ensure that also deactivated benzoates and heteroaromatic carboxylic esters condense without appreciable formation of side products (see table 2). As expected, carboxylic esters with α-hydrogens (**2g**) are unsuited, and also weakly acidic heteroaromatic compounds (**2k**) cannot be applied. The same is the case for nitro derivatives which are prone to reduction. Noticeably, the series of successful conversions to the diketone included a deactivated ester (**2b**), as well as a bifunctional building block and a component with an additional electrophilic center (**2e** and **2f** resp.).

With regards to the α-alkylation step, we obtain best results in the presence of TBAF, which has the function to shield the oxygen atoms of the β-dicarbonyl intermediate, hence inhibiting O-alkylation as a side reaction and furthermore increasing the nucleophilicity of the compound. Water traces are detrimental to the yield, which otherwise lies around 90% of the C-monoalkylated product. To expand the diversity, alkylating agents other than the simple alkyl iodides described in analogous solution chemistry can be used (Clark *et al.*, J. Chem Soc., Perkin Trans. I (1977), 1743). Ethyl bromoacetate and allyl bromide react without side reactions. With iodoacetonitrile 35% of starting material is observed and bromoacetophenone does not convert cleanly. The failure with benzyl bromide is rather unexpected. The alkylation step is incompatible with the presence of acid or basic heteroaromatic R^1 and R^2 residues: with the phenyl pyridine diketone **2j** several side products are observed upon alkylation with all the listed alkylating agents. Naturally, dispensing with the alkylation altogether enables to broaden the choice of building blocks for the previous Claisen condensation by allowing e.g. the inclusion of N-heterocyclic esters as an alternative source of diversity.

Ring closure to form a heterocyclic scaffold is tested successfully with hydrazines **4a-d** and hydroxylamine (**5a**) (see table 4). With the non-alkylated intermediate **2a** a faster cyclization kinetics than with **3a** is observed. N-mono-substituted reagents are expected to yield regioisomers with equal efficiency, unless the intermediates would bear large differences of steric and electronic properties at the R^1 and R^2 sites.

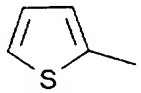
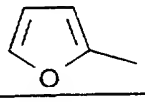
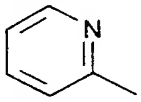
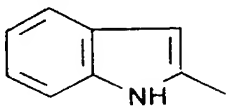
During the validation process we isolate both regioisomers of model compound **4d**. In this instance we intentionally explore the limits by choosing a difficult case, since hydralazine (for electronic and steric reasons) is predicted to have a relatively weak tendency to cyclize. In fact, after 1 day, only traces of the regioisomers **4d** are detectable, and it takes 4 days to obtain a level of 20 % conversion, in the presence of the unsubstituted analog **4a** originating from the far more reactive impurity **2a** (the non-alkylated diketone precursor).

Table 1. Coupling procedure to acetyl compounds **1a-c**

	R^1	loading[1)]		R^1	loading[1)]
1a	4-C_6H_4	424			
1b		410	1c		-[2)]

1) resin capacity [µmol/g] 2) intramolecular ring closure reaction

Table 2. Claisen condensation to β-diketone **2a-k** (R^1 = 4-C_6H_4)

	R^2	yield[1)]		R^2	yield[1)]
2a	C_6H_5	100%	2h		100%
2b	4-$CH_3OC_6H_4$	100%	2i		95%
2c	4-ClC_6H_3(2-NO_2)	-[2)]	2j		100%
2d	4-ClC_6H_3(2-Cl)	100%	2k		-[4)]
2e	4-$CH_3OOCC_6H_4$	100%			
2f	4-NCC_6H_4	100%			
2g	CH_3	-[3)]			

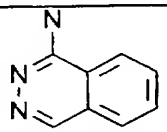
1) by HPLC 2) is prone to reduction
3) multicomponent mixture 4) formation of indolyl anion

Table 3. α-Alkylation of diketone **2a** (R^1 = 4-C_6H_4, R^2 = C_6H_5)

	R^3	X	yield[1)]		R^3	X	yield[1)]
3a	CH_3CH_2	I	90%	3d	$NCCH_2$	I	60%
3b	$C_6H_5CH_2$	Br	0%	3e	$C_6H_5COCH_2$	Br	65%
3c	$CH_3CH_2OOCCH_2$	Br	90%	3f	CH_2=$CHCH_2$	Br	90%

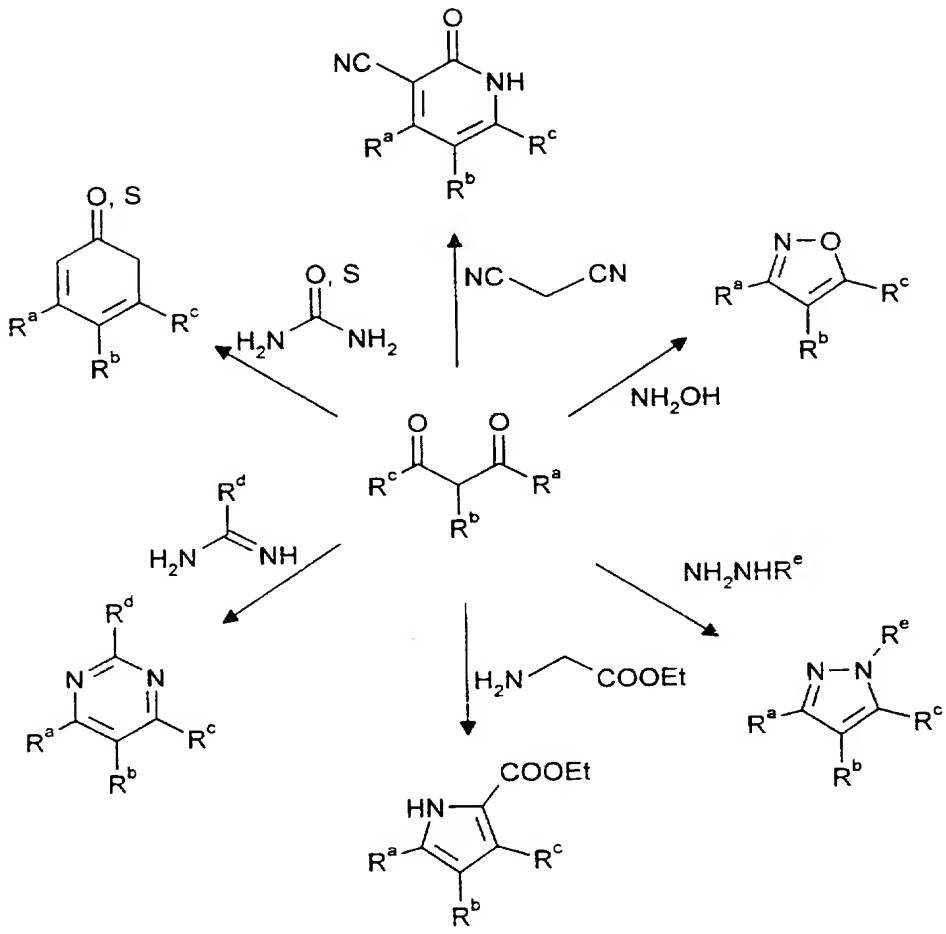
1) by HPLC

Table 4. Formation of pyrazoles **4a-d** (R^1 = 4-C_6H_4, R^2 = C_6H_5, R^3 = CH_3CH_2) and isoxazole **5a** (R^1 = 4-C_6H_4, R^2 = C_6H_5, R^3 = CH_3CH_2)

	Y	yield[1)]		Y	yield[1)]
4a	NH	100%	5a	O	100%
4b	NCH_3	100%			
4c	NC_6H_5	100%			
4d		20%			

1) by HPLC of regioisomers

The collected data on the conversion rates of a variety of reactants within our proposed synthetic scheme provide an information basis sufficient for the planning of combinatorial libraries along different lines of strategies. Decisions can be made on which types of building blocks to include for diversity generation and whether to skip a reaction step in favor of a broader choice of compatible residues or simplified reaction conditions. Moreover, the general utility of the approach could be exploited to form additional ring types (e.g. pyrimidines from amidines see formula 5) if the diketone intermediates were subjected to cyclization with other reagents bearing two nucleophilic centers.



(5)

General method for the synthesis:

a) Loading of a solid carrier with an acetyl function

4-(2',4'-Dimethoxyphenyl-fmoc-aminomethyl)phenoxy resin (Rink amide resin) is subjected to repeated ishes with 20% piperidine/DMF until no UV absorption from Fmoc is detected in the eluate.

b) Coupling procedure:

The NH_2-linker group is acylated with 0.3 M-solution of acetyl carboxylic acid (3 eq) at RT (preactivation 40 min with 3.3 eq DICD and 3.3 eq HOBt) until the Kaiser test (Kaiser *et al.*, Anal. Biochem. (1970), **34**, 595) is negative.

c) Claisen condensation

50 mg (22.5 μmol) of the modified resin are suspended in a solution of 675 μmol carboxylic ester in 670 μl DMA. Under inert gas 18 mg (450 μmol) of sodium hydride (60% dispersion in mineral oil) is added and the reaction mixture is well shaken for 1h at 90 °C. The resin is filtered, ished (30% v/v acetic acid / H_2O, DMA, DMSO, and i-propanole), and dried under reduced pressure.

d) α-Alkylation step (if necessary)

20 mg (8.6 μmol) of this modified resin is treated with 86 μl 1M TBAF in THF for 2h at room temperature. After addition of 150 μl of a 2.5 M solution of the appropriate alkylating agent, the reaction is continued for another 2 h. The resin is filtered off and ished well with CH_2Cl_2 and THF.

e) Ring closure

The resin resulting from these modifications is heated with 500 μl of a 2.5 M solution of hydrazine derivatives or hydroxyl amine (HCl is neutralized by $N(CH_3CH_2)_3$) in DMA for 24 h at 80°C.

f) Release of the compound from the solid carrier

Cleavage from the support is done by with 20% v/v TFA/CH_2Cl_2 (Rink, Tetrahedron Lett. (1987), **28**, 3787).

A suitable method for the preparation of a combinatorial compound library comprises, for example, the reaction steps as described above, wherein optionally before a reaction step is carried out,

a) the resin pool is divided into different portions,
b) said reaction step is carried out in each portion using a different chemical compound or reaction, and
c) the portions are mixed together.

Alos embraced by the scope of the current invention is the combinatorial compound library obtainable by the method described above.

In order to synthesize a combinatorial compound library, e.g. according to Houghten *et al.* Nature (1991) **354**, 84-86; a solid carrier having reactive surface groups is loaded with a compound bearing an acetyl function; or the resin is divided first into several portions then loaded with a different compound bearing an acetyl function in each portion and mixed again. Afterwards, e.g., the pool containing the modified resin is divided into several separate portions again. The Claisen condensation is carried out in each portion using a different reagent to get different compounds. These separated pools are mixed and, if appropriate, divided again into several separate portions in which the optional α-alkylation step using different reagents is carried out and, afterwards, the separated pools are mixed again. If desired, the mixture may be divided into several separate portions again for carrying out the ring closure with a different agent in each portion. After mixing, a combinatorial compound library has been created that is suitable, e.g., for screening.

Abbreviations

HOBt = 1-hydroxybenzotriazole
DICD = diisopropylcarbodiimide
DMA = dimethylacetamide
DMF = dimethylformamide
DMSO = dimethylsulfoxide
Fmoc = fluorenylmethyloxycarbonyl
TBAF = tetra-n-butylammonium fluoride
TFA = trifluoroacetic acid
THF = tetrahydrofuran

Examples

The products that are produced in each step are analyzed after cleavage from the solid carrier by the following method:

Cleavage from the support is done by with 20% v/v TFA/CH_2Cl_2 (Rink, Tetrahedron Lett. (1987), **28**, 3787).

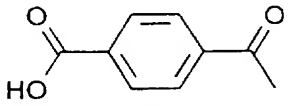
HPLC analytical separation is achieved using a reverse phase nucleosil C18 5μ 250 mm × 4.6 mm column, 215 nm, 10-90% CH_3CN / 0.1% TFA over 30 min, 1 ml/min.

A part of the eluate (split 1:25) is introduced into a Quattro-BQ mass spectrometer (VG Biotech, Altrincham, England), operating at a source temperature of 60°C and a cone voltage of 50 V, via an electrospray interface (EI). The mass range from 100 to 800 Dalton is scanned in 4 seconds.

Example 1 : Loading of the resin with a compound bearing an acetyl function

Deprotection: 4-(2',4'-Dimethoxyphenyl-fmoc-aminomethyl)phenoxy resin (Rink amide resin) is subjected to repeated washes with 20% piperidine/DMF until no UV absorption from Fmoc is detected in the eluate.

Coupling procedure: The NH_2-linker group is acylated with 0.3 M-solution of 3 eq of a compound of formula 6



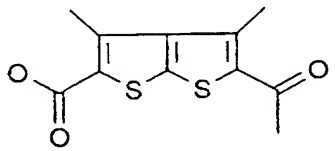
(6)

at RT (preactivation 40 min with 3.3 eq DICD and 3.3 eq HOBt) until the Kaiser test (Kaiser, E. et al. *Anal. Biochem.* **1970**, *34*, 595) is negative. The loading of the resin is 424 μmol/g.

Example 2 : Alternative loading of the resin

Deprotection: 4-(2',4'-Dimethoxyphenyl-fmoc-aminomethyl)phenoxy resin (Rink amide resin) is subjected to repeated washes with 20% piperidine/DMF until no UV absorption from Fmoc is detected in the eluate.

Coupling procedure: The NH_2-linker group is acylated with 0.3 M-solution of 3 eq of a compound of formula 7

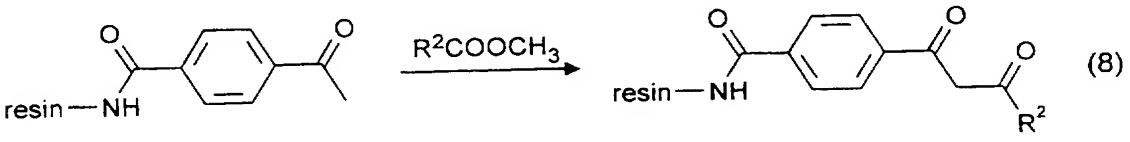


(7)

at RT (preactivation 40 min with 3.3 eq DICD and 3.3 eq HOBt) until the Kaiser test (Kaiser, E. et al. *Anal. Biochem.* **1970**, *34*, 595) is negative. The loading of the resin is 410 (resin capacity in [μmol/g]).

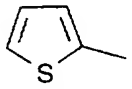
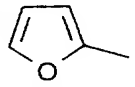
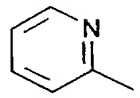
Example 3 : Claisen condensations

50 mg (22.5 µmol) the modified resin of example 1 are suspended in a solution of 675 µmol carboxylic ester in 670 µl DMA (see table 5). Under inert gas 18 mg (450 µmol) of sodium hydride (60%) is added and the reaction mixture is well shaken for 1h at 90 °C. The resin is filtered, ished (30% v/v acetic acid / H_2O, DMA, DMSO, and i-propanole), and dried under reduced pressure.



(8)

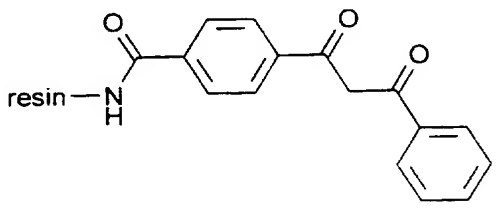
Table 5. Claisen condensation to β-diketone according to formula 8

R^2	yield [%]	R^2	yield [%]
C_6H_5	100	4-NCC_6H_4	100
4-$CH_3OC_6H_4$	100		100
4-ClC_6H_3(2Cl)	100		95
4-$CH_3OOCC_6H_4$	100		100

The yields are calculated from the HPLC purification

Example 4 : Heterocyclic compounds with two or three substituents

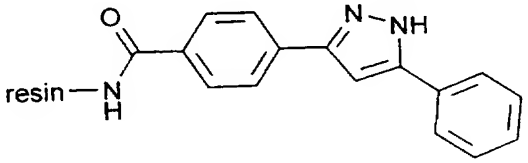
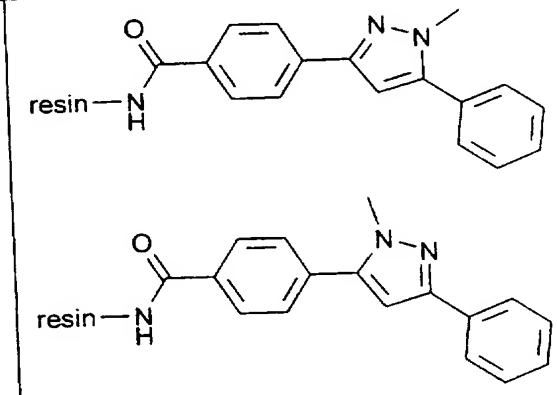
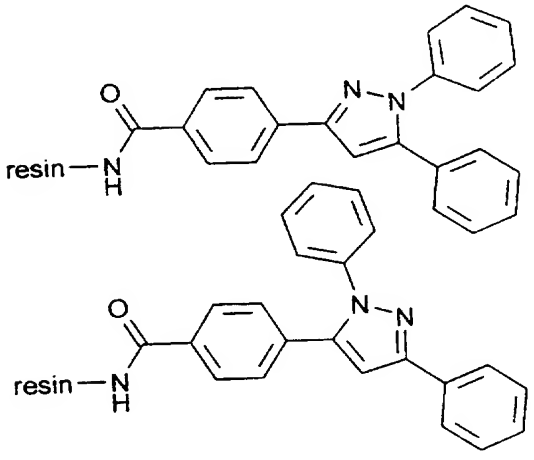
50 mg (22.5 µmol) modified resin of example 1 are suspended in a solution of 675 µmol methylbenzoate in 670 µl DMA. Under inert gas 18 mg (450 µmol) of sodium hydride (60%) is added and the reaction mixture is well shaken for 1h at 90 °C. The resin is filtered, ished (30% v/v acetic acid / H_2O, DMA, DMSO, and i-PrOH), and dried under reduced pressure to give a compound of formula 9.

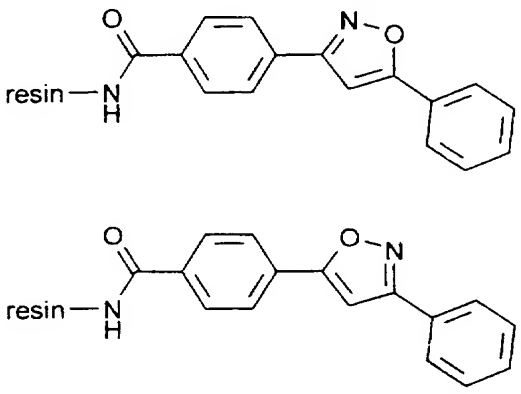
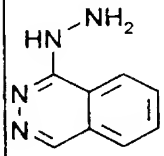
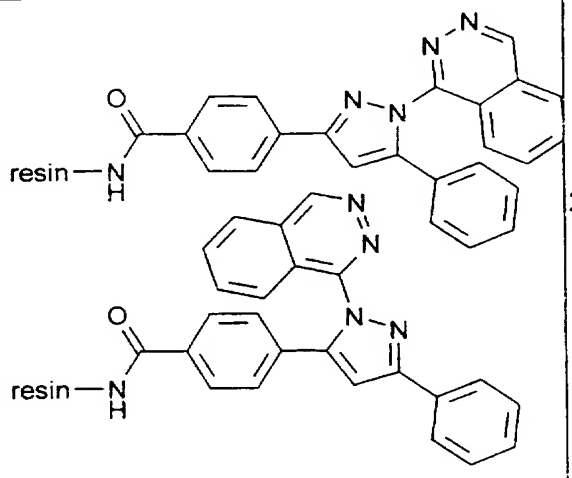


(9)

This modified resin is heated with 500 µl of a 2.5 M solution of a nucleophile (HCl is neutralized by $N(CH_3CH_2)_3$) in DMA for 20-24 h (see table 6) to give the desired heterocyclic products (see table 6).

Table 6

nucleophile	heterocyclic products	yield [%]	R_t of the products [min]
NH_2NH_2		100	20.9
NH_2NHCH_3		100	22.4 and 22.8
$NH_2NHC_6H_5$		100	27.2 and 27.6

NH_2OH		100	24.2 and 24.6
		20	23.3 and 24.7

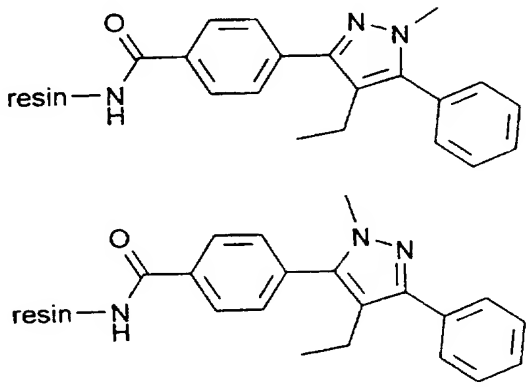
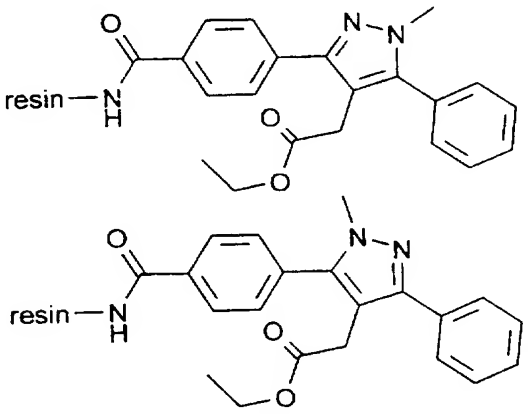
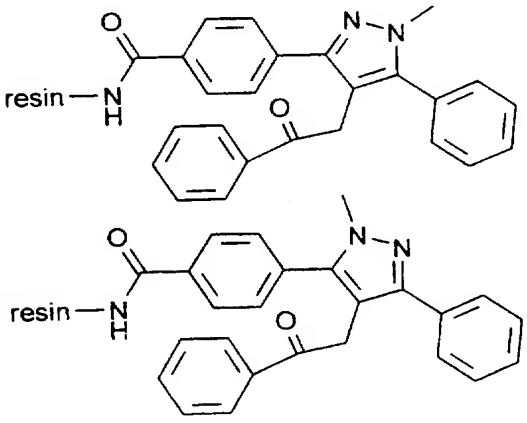
The products have been analyzed using HPLC after cleavage from the resin as described above.

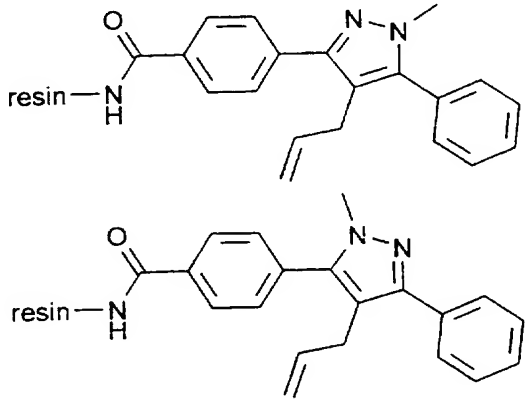
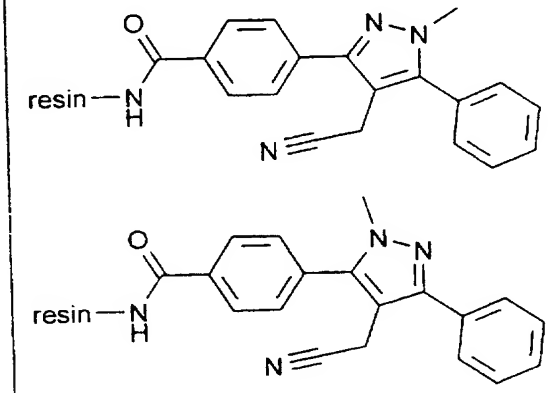
Example 5 : Heterocyclic compounds with four substituents

50 mg (22.5 µmol) modified resin of example 1 are suspended in a solution of 675 µmol methylbenzoate in 670 µl DMA. Under inert gas 18 mg (450 µmol) of sodium hydride (60%) is added and the reaction mixture is well shaken for 1h at 90 °C. The resin is filtered, ished (30% v/v acetic acid / H_2O, DMA, DMSO, and i-propanole), and dried under reduced pressure. 20 mg (8.6 µmol) of this modified resin is treated with 86 µl 1M TBAF in THF for 2h at room temperature. After addition of 150 µl of a 2.5 M solution of the appropriate alkylating agent (see table 7), the reaction is continued for another 2 h. The resin is filtered off and ished well with CH_2Cl_2 and THF. Than the modified resin is heated with 500 µl of a

50 % solution of methylhydrazine (HCl is neutralized by $N(CH_3CH_2)_3$) in ethanol for 16 h at 56°C to give the desired heterocyclic products (see table 7).

Table 7

alkylating agent	heterocyclic products	yield [%]	R_t of the products [min]
CH_3CH_2I		90	24.1 and 24.9
$CH_3CH_2OOCCH_2Br$		90	18.3 and 18.9
$C_6H_5COCH_2Br$		65	24.6 and 25.1

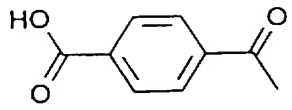
$H_2C=CHCH_2Br$		90	24.8 and 25.4
$NCCH_2I$		60	

The products have been analyzed using HPLC after cleavage from the resin as described above.

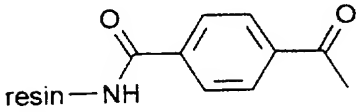
Example 6 : Ring closure with compound that has a weak tendency to cyclize

Deprotection: 4-(2',4'-Dimethoxyphenyl-aminomethyl)phenoxy resin (Rink amide resin) is subjected to repeated washes with 20% piperidine/DMF until no UV absorption from Fmoc is detected in the eluate.

The NH_2-linker group of 1.50 g 4-(2',4'-Dimethoxyphenyl-fmoc-aminomethyl)phenoxy resin (Rink amide resin) is acylated with a 0.3 M-solution of a compound of formula 10 (3 eq) at RT (after preactivation for 40 min with 3.3 eq DICD and 3.3 eq HOBt) until the Kaiser test (Kaiser, E. et al. *Anal. Biochem.* **1970**, *34*, 595) is negative to form a compound of formula 11.



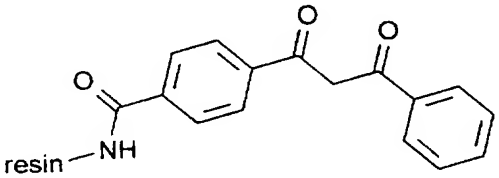
(10)



(11)

A portion of the product is cleaved from the resin as defined above and analyzed: 100% purity by HPLC, 10.2 min, MS (EI) m/z 163 (M^+).

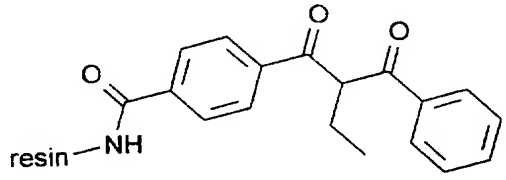
50 mg (22.5 µmol) of the compound of formula 11 are suspended in a solution of 0.51g (3.4 mmol) $C_6H_5COOCH_2CH_3$ in 670 µl DMA. Under inert gas 0.14 g (3.4 mmol) NaH (60% dispersion in mineral oil), and 10.5 ml DMA ;and the reaction mixture is well shaken for 1h at 90 °C. The resin is filtered, ished (30% v/v acetic acid / H_2O, DMA, DMSO, and i-propanole), and dried under reduced pressure to get a compound of formula 12.



(12)

A portion of the product is cleaved from the resin as defined above and analyzed: 95% purity by HPLC, 26.3 min, MS (EI) m/z 267 (M^+).

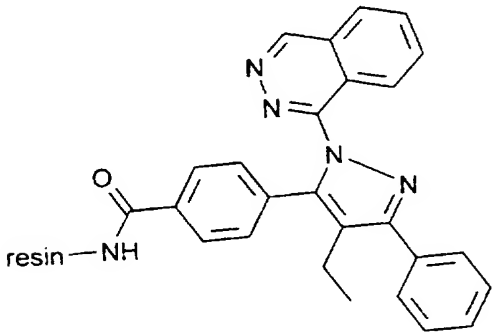
20 mg (8.6 µmol) of this resin is treated with 86 µl 1M TBAF in THF for 2h at room temperature. After addition of 150 µl of a 2.5 M of CH_3CH_2I in CH_2Cl_2, the reaction is continued for another 2 h. The resin is filtered off and ished well with CH_2Cl_2 and THF to give a compound of formula 13.



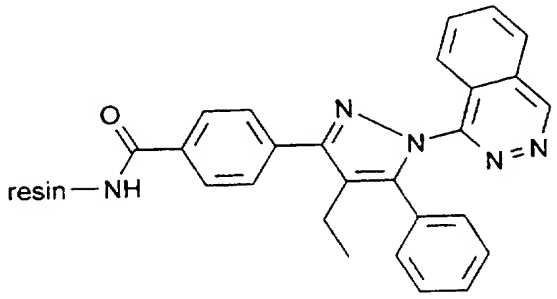
(13)

A portion of the product is cleaved from the resin as defined above and analyzed: 75% purity by HPLC, 22.7 min, MS (EI) m/z 294 (M^+).

The compounds of formulas 13 is heated for 4 days under reflux with 1.09 g (6.75 mmol) hydralazine and 67 mg (0.67 mmol) acetylacetone in CH_3CH_2OH to form of the two isomeres of formulas 14 and 15.



(14)



(15)

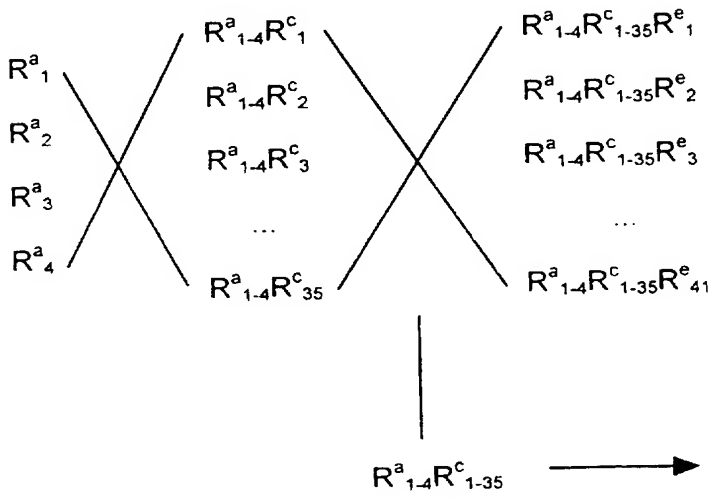
For analyzation the product is cleaved from the resin with 20% v/v TFA/CH_2Cl_2 as defined above.

HPLC preparative separation is achieved using a reverse phase nucleosil C18 5μ 20 mm × 250 mm column, 215 nm, 10-90% CH_3CN/0.1% TFA over 90 min, 15 ml/min (10% purity by HPLC, 25.3 min for isomer 15 and 26.3 min for isomer 14)

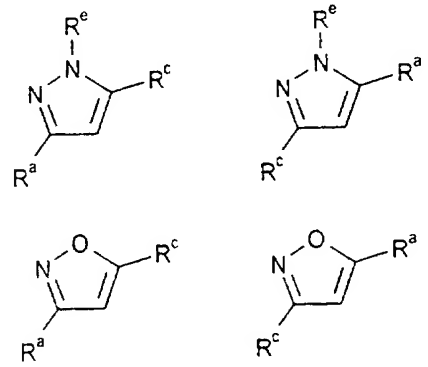
isomer 15: ^{1}H-NMR (DMSO-d_6) δ 9.72 (s, 1H), 8.29 (m, 1H), 8.11 (m, 4H), 8.02 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.6 Hz, 2H) 7.46 (bs, 1H), 7.32 (m, 2H), 7.24 (m, 1H), 2.77 (q, J = 7.4 Hz, 2H), 1.06 (t, J = 7.4 Hz, 3H), MS (EI) m/z 420 (M^+).

isomer 14: ^{1}H-NMR (DMSO-d_6) δ 9.71 (s, 1H), 8.29 (m, 1H), 8.20 (m, 1H), 8.15 (m, 2H), 7.99 (bs, 1H), 7.82 (m, 4H), 7.51 (m, 4H), 7.42 (bs, 1H), 7.31 (d, J = 8.5 Hz, 2H), MS (EI) m/z 420 (M^+).

Example 7 : Pyrazole/Isoxazole library

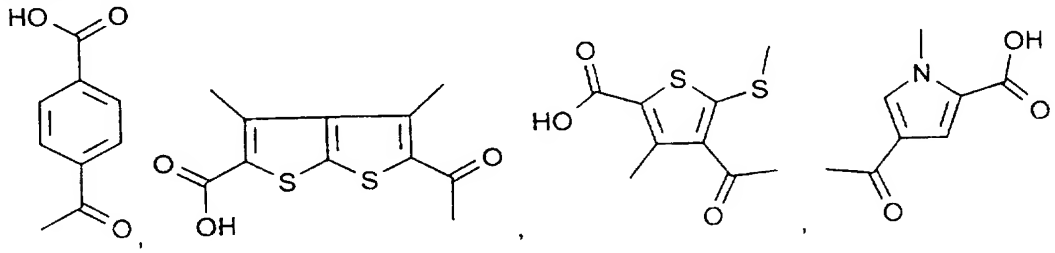


randomized pyrazole library
11480 compounds divided into
41 sublibraries with
280 components
(140 pairs of regioisomeres)

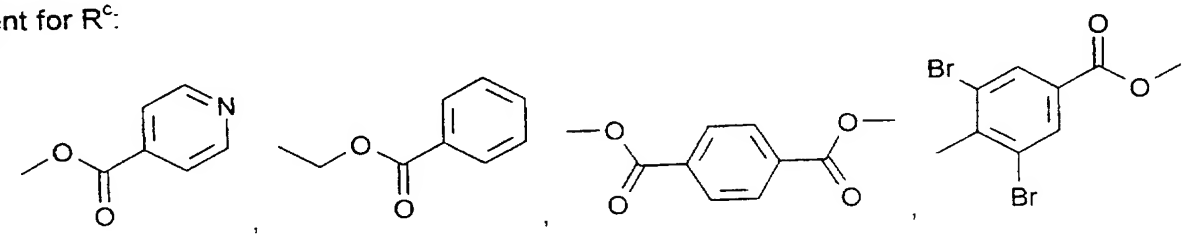


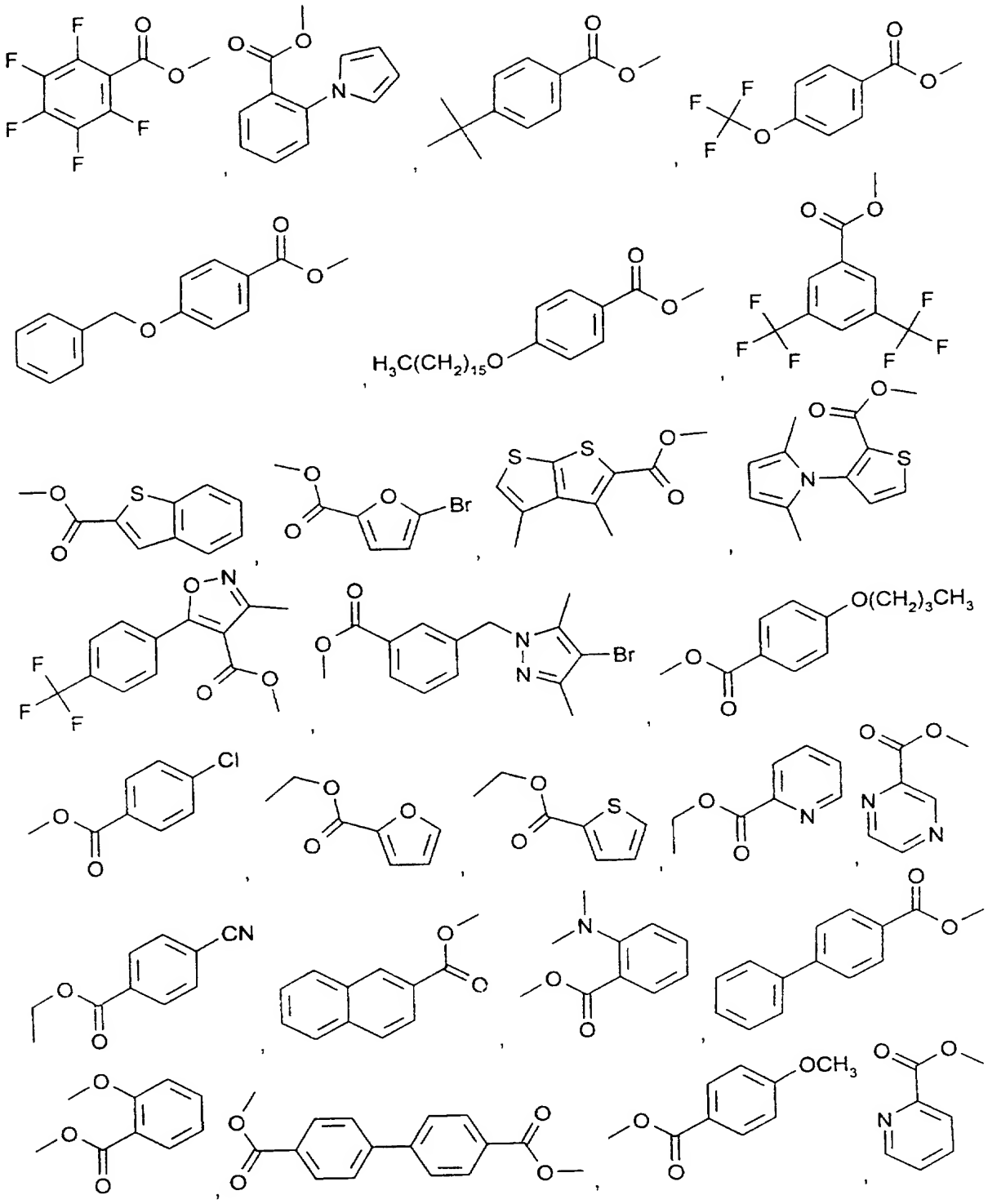
In a first step the acetyl carboxylic acids R^a_1 to R^a_4 are coupled to the resin in separate vessels. The four portions are mixed and distributed in 35 vessels, where each portion reacted with a distinct R^c reagent, a carboxylic acid ester. The result is a randomized R^a and a fixed R^c position. The beads are mixed again and divided into 42 equal portions, of which 41 are reacted with monosubstituted hydrazines R^e. We get a pyrazole library with 11480 compounds divided into 41 sublibraries with 280 compounds each. The remaining last portion of the beads is reacted with hydroxylamine to an isoxazole library comprising 280 compounds. The reagents are used are defined below.

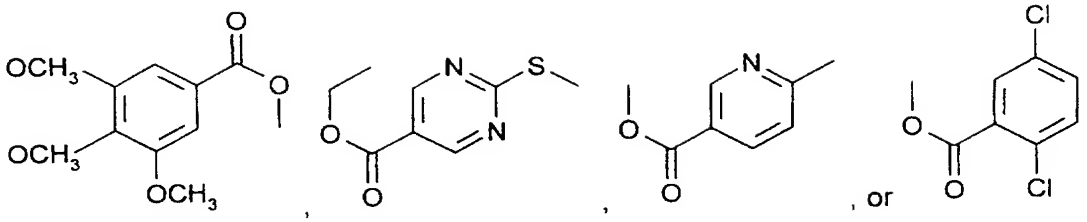
Agent for R^a:



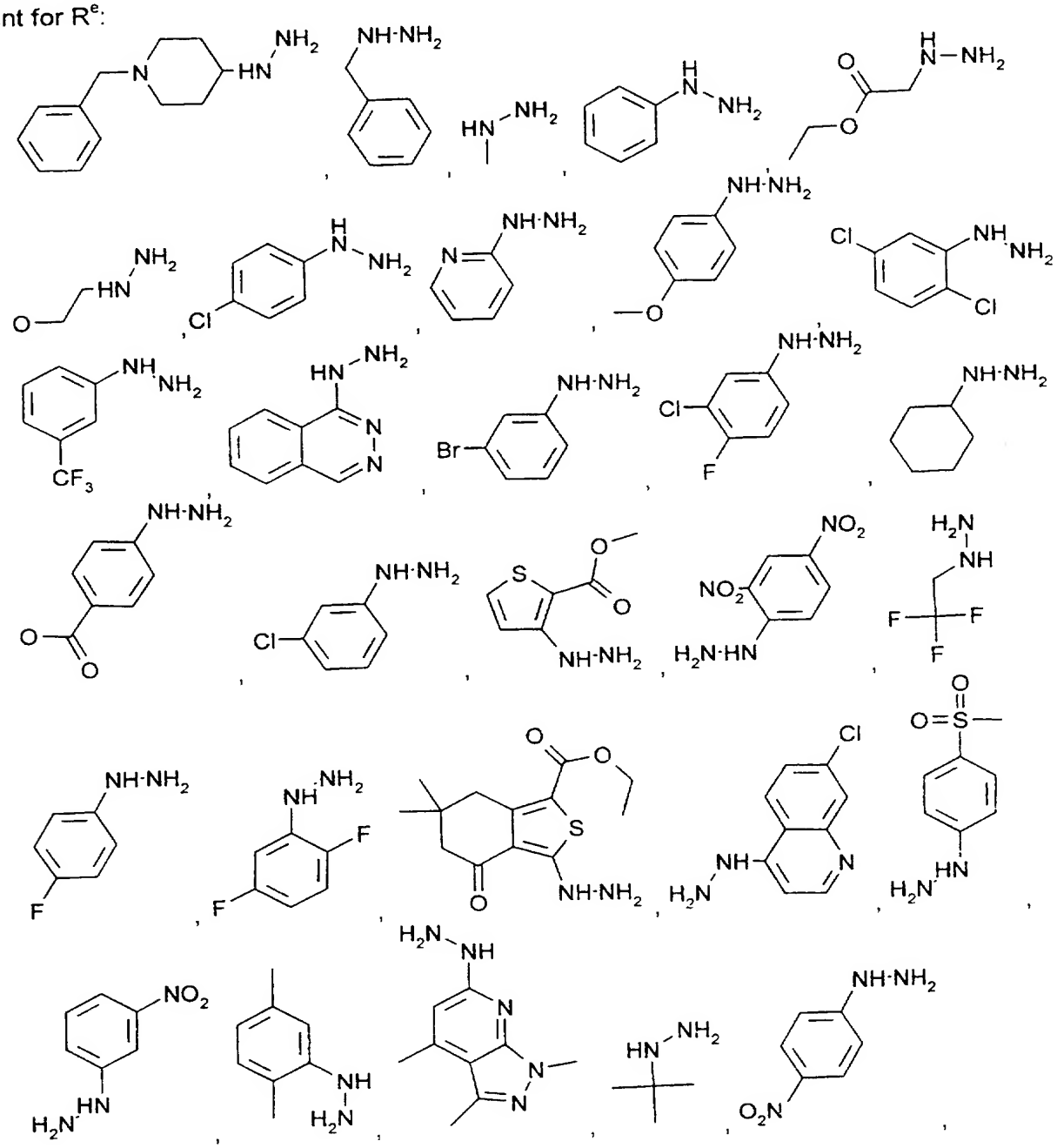
Agent for R^c:

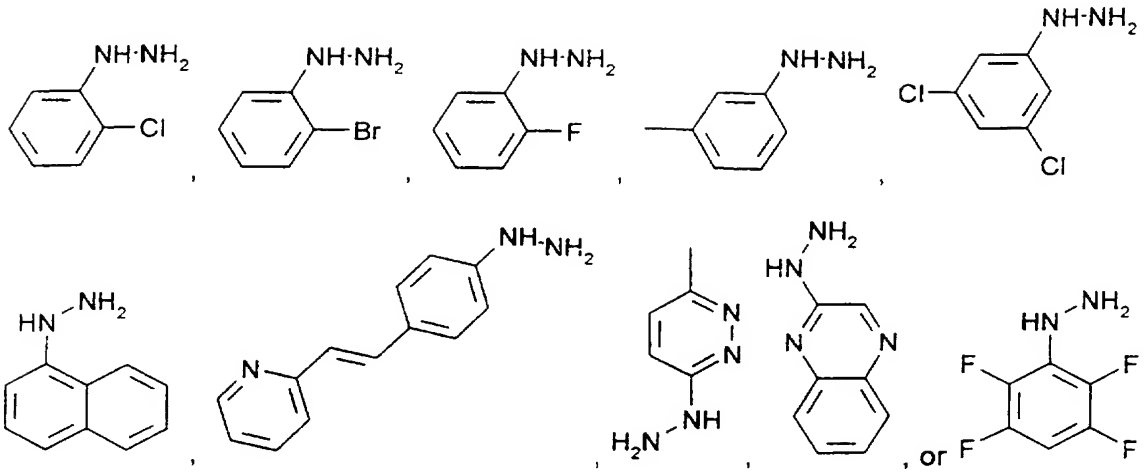






Agent for R^e:





Library Synthesis

Coupling of 4 acetyl carboxylic acids to Rink amide resin: Four portions, of Rink amide resin each 0.5 g (208.5 µmol) are treated with 3 eq of a 0.3 M solution of the appropriate carboxylic acid (agent for R^a) which is preactivated with 3.3 eq DICD and HOBt for 40 minutes. After the Kaiser test is negative the four portions are mixed and washed with DMA, DMSO, and i-PrOH and dried under vacuo.

Claisen Condensation: The resin from the former coupling procedure is divided in 35 separate reaction vessels. Under inert gas atmosphere 21 mg (521 µmol) sodium hydride and 782 µmol carboxylic acid ester (agent for R^c)in 770 µl DMA are added to each resin portion (23.4 µmol). The vigorously mixed reaction mixtures are heated 75 min at 90°C. All portions are mixed and washed with 30 % v/v acetic acid/water, THF, DMA, DMSO, i-PrOH and dried under vacuo.

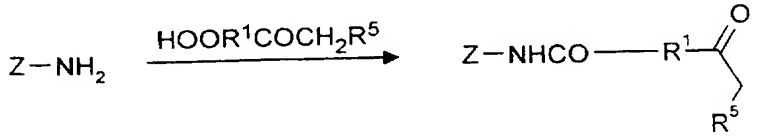
Cyclization: 1.6 g (640 µmol) are separated in 42 reaction vessels and each resin is treated with 790 µl of a 0.5 M solution of the appropriate monosubstituted hydrazine in DMA (agent for R^e). After heating the reaction mixtures for 3 days at 90°C each portion is washed separately with DMA, DMSO , and i-PrOH.

Cleavage: Approx. 1/3 of the library material, i. e. 12.07 mg (5 µmol) resin from each sublibrary are mixed with 300 µl 20 % v/v TFA/CH_2Cl_2 three times for 30 minutes. Then the resin is washed with 300 µl 1,2-dichloroethane and 300 µl trifluoroethanol. The solvents are evaporated in a microcentrifuge and the residue is dissolved in 500 µl DMSO.

Claims

1. Solid phase synthesis of a heterocyclic ring, characterized in that it comprises the following steps:
   a) a solid carrier having reactive surface groups is loaded directely or via a spacer group with a compound bearing an acetyl function,
   b) said acetyl function is modified using the Claisen condensation,
   c) optional the reaction product of step b) is modified with an α-alkylation step, and
   d) the heterocyclic ring is closed using a compound comprising two nucleophiles, wherein at least one of said nucleophiles is NH_2.

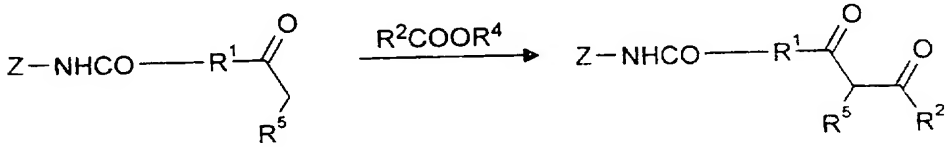
2. Solid phase synthesis according to claim 1, characterized in that step a) is of formula 1



(1)

wherein
Z is a solid carrier;
R^1 is a substituted or unsubstituted conjugated system that has no acidic hydrogen atoms; and
R^5 is hydrogen, C_1-C_6alkyl, C_1-C_6alkylaryl, C_1-C_6alkoxy-C_1-C_6alkyl, C_1-C_6alkoxycarbonyl-C_1-C_6alkylene; each of which may be substituted or unsubstituted.

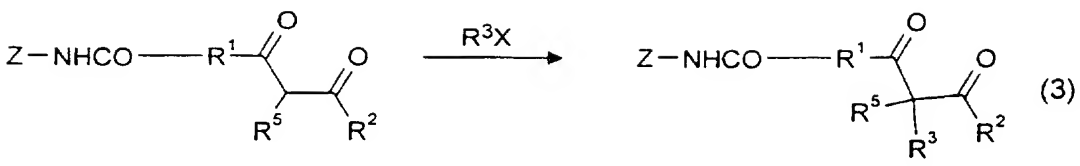
3. Solid phase synthesis according to claim 1, characterized in that step b) is of formula 2



(2)

wherein
Z, R^1 and R^5 are defined as in claim 2;
R^2 is substituted or unsubstituted C_1-C_6alkyl, or a substituted or unsubstituted aromatic or aliphatic ring; and
R^4 is C_1-C_6alkyl.

4. Solid phase synthesis according to claim 1, characterized in that step c) is of formula 3



(3)

wherein

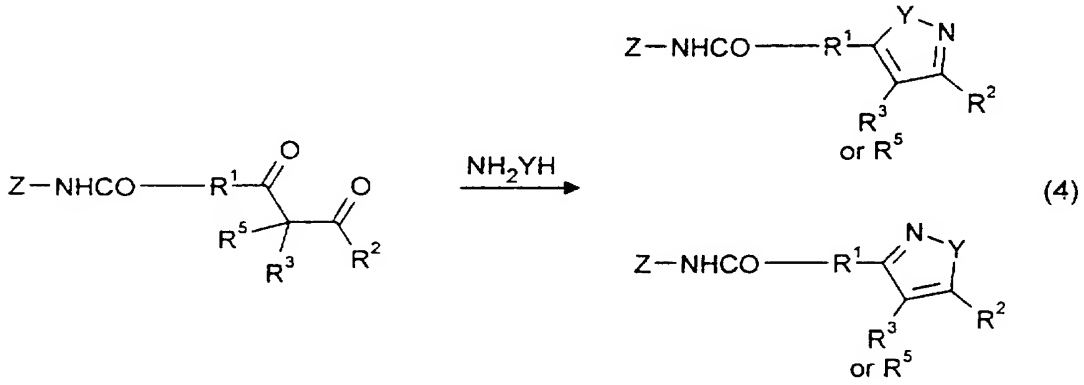
Z, R^1, and R^2 are defined as in claim 3;

R^5 is hydrogen

X is a halogen; and

R^3 is C_1-C_6alkyl, C_1-C_6alkenyl, C_1-C_6alkylcarbonyl-C_1-C_6alkylene, arylcarbonyl-C_1-C_6alkylene, or a substituted or unsubstituted aromatic or aliphatic ring.

5. Solid phase synthesis according to claim 1, characterized in that step d) is of formula 4



(4)

wherein

Z, X, R^1, R^2, R^3, R^4 and R^5 are defined as above;

Y is a nucleophilic center;

with the proviso that either R^3 or R^5 is hydrogen.

6. Use of a solid phase synthesis according to claim 1 for the generation of combinatorial compound libraries.

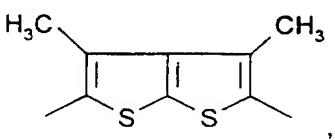
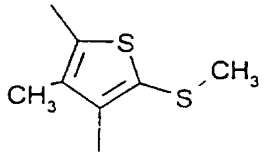
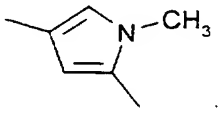
7. Method for the preparation of a combinatorial compound library, which comprises the steps according to claim 1, wherein optionally before a reaction step is carried out,

a) the resin pool is divided into different portion,

b) *said reaction* step is carried out in each portion using a different chemical compound or reaction, and

c) the portions are mixed together.

8. Solid phase synthesis according to claim 1, characterized in that Z is a resin.

9. Solid phase synthesis according to claim 2, characterized in that R^1 is ethinylene, thienylene, thiantrenylene, furylene, phenoxanthiinylene, isobenzofuranylene, pyrazolylene, isothiazolylene, isoxazolylene, pyridinylene, pyrazinylene, pyrimidylene, indolizinylene, indazolylene, isoquinolylene, quinolylene, phthalazinylene, naphthyridinylene, quinoxalinylene, quinazolylene, cinnolinylene, phenylene, naphthylene, naphthylene,  ,  ,or  ; wherein these conjugated systems are unsubstituted or substituted by groups that have no acidic hydrogens.

10. Solid phase synthesis according to claim 3, characterized in that R^2 is ethinyl, thienyl, thiantrenyl, furyl, phenoxanthiinyl, isobenzofuranyl, pyrazolyl, isothiazolyl, isoxazolyl, pyridinyl, pyrazinyl, pyrimidyl, indolizinyl, indazolyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolyl, cinnolinyl, phenyl and naphthyl; wherein these conjugated systems are unsubstituted or substituted by groups that have no acidic hydrogens.

11. Solid phase synthesis according to claim 4, characterized in that R^3 is hydrogen, C_1-C_6alkyl, C_1-C_4alkoxy-C_1-C_4alkyl, C_1-C_6alkenyl, C_1-C_4alkoxy-aryl, C_1-C_5alkanoyloxy-C_1-C_6alkyl, C_1-C_6alkoxycarbonyl-C_1-C_5alkyl.

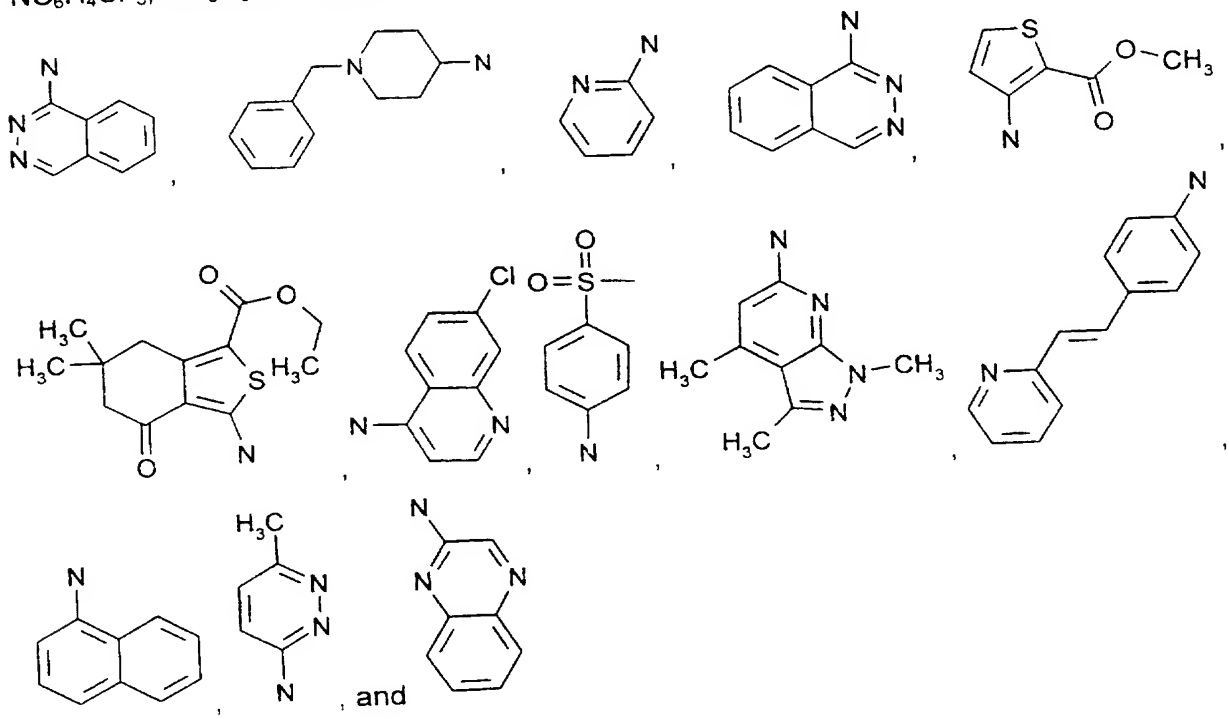
12. Solid phase synthesis according to claim 3, characterized in that R^4 is hydrogen, methyl, ethyl

13. Solid phase synthesis according to claim 4, characterized in that X is I, Br or Cl.

14. Solid phase synthesis according to claim 5, characterized in that Y is O, NH, NC_1-C_4alkyl, NC_1-C_4alkyl aryl, Naryl unsubstituted or substituted with up to four Br, Cl, I, F, C_1-C_4alkyl, NO_2, SO_2C_1-C_4alkyl, OC_1-C_4alkyl, carboxy or carbonyl groups.

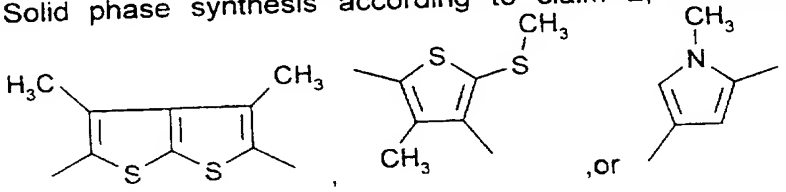
15. Solid phase synthesis according to claim 5, characterized in that Y is NH, O, NCH_3, NC_6H_5, $NCH_2C_6H_5$, $NCH_2COOC_2H_5$, NC_6H_4Cl, $NC_6H_3Cl_2$, NC_6H_4F, $NC_6H_3F_2$, NC_6HF_4,

NC_6H_4Br, $NC_6H_4CH_3$, $NC_6H_3(CH_3)_2$, NC_2H_4OH, $NC_6H_4OCH_3$, $NC_6H_3Cl_2$, NC_6H_4Br, $NC_6H_4CF_3$, NC_6H_3ClF, NC_6H_{10}, NC_6H_4COOH, $NC_6H_3(NO_2)_2$, $NC_6H_4NO_2$, NCF_3, $NC(CH_3)_3$,

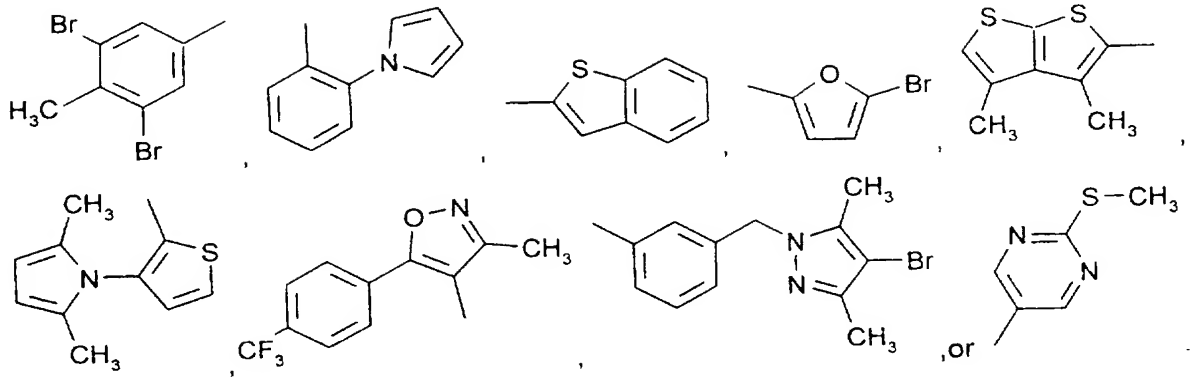


16. Solid phase synthesis according to claim 2, characterized in that Z is a particle that is insoluble in the reaction media and to which the ligand can be bound in sufficient amount by means of reactive groups at the surface of the this particle.

17. Solid phase synthesis according to claim 2, characterized in that R^1 is benzene,



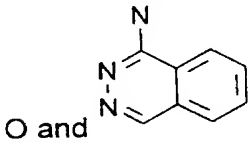
18. Solid phase synthesis according to claim 3, characterized in that R^2 is phenyl, 4-$CH_3OC_6H_4$, 4-$ClC_6H_3(2Cl)$, 4-$CH_3OOCC_6H_4$, 4-NCC_6H_4, furyl, pyrrolyl, thienyl, pyridyl, methyl pyrridyl, pyrazinyl, $C_6H_5COOCH_3$, C_6F_5, $C_6H_4C(CH_3)_3$, $C_6H_4OCF_3$, $C_6H_4OCH_2C_6H_5$, $C_6H_4O,(CH_2)_{15}CH_3$, $C_6H_3(CF_3)_2$, $C_6H_4O(CH_2)_3CH_3$, C_6H_4Cl, C_6H_4CN, naphthyl, $C_6H_4N(CH_3)_2$, $C_6H_4C_6H_5$, $C_6H_4OCH_3$, $C_6H_4C_6H_4COOCH_3$, $C_6H_2(OCH_3)_3$, $C_6H_3Cl_2$,



19. Solid phase synthesis according to claim 4, characterized in that R^3 is hydrogen, ethyl, $NCCH_2$, $CH_3CH_2OOCCH_2$, $C_6H_5COCH_2$, $CH_2=CHCH_2$.

20. Solid phase synthesis according to claim 4, characterized in that R^4 is hydrogen, methyl, ethyl

21. Solid phase synthesis according to claim 5, characterized in that Y is NH, NCH_3, NC_6H_5,



22. Solid phase synthesis according to claim 2, characterized in that Z is a particle that is insoluble in the reaction media and to which the ligand can be bound in sufficient amount by means of reactive groups at the surface of the this particle.

23. A combinatorial compound library obtainable by a method according to claim 7.

24. Use of the combinatorial compound library according to claim 23 for screening.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/[illegible] 96/04808

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07B61/00 C07D231/12 C07D261/08 C07D403/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07B C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

	Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
8	X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 90, no. 15, 1 August 1993, WASHINGTON US, pages 6909-6913, XP002025140 S. HOBBS DEWITT ET AL.: ""Diversomers": An approach to nonpeptide, nonoligomeric chemical diversity" see the whole document ---	23,24
6	X	US 5 288 514 A (ELLMAN JONATHAN A) 22 February 1994 see the whole document ---	23,24
3	Y	US 3 948 937 A (JOHNSON ALEXANDER LAWRENCE ET AL) 6 April 1976 see column 4, line 9 - line 40 see column 14 - column 15; examples 17-45 ---	1-22
		-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
12 February 1997	03. 03. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Fink, D

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

	Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
7	Y	TETRAHEDRON, (INCL TETRAHEDRON REPORTS), vol. 51, no. 30, 24 July 1995, OXFORD GB, pages 8135-8173, XP002025141 N.K. TERRETT ET AL.: "Combinatorial Synthesis - The Design of Compound Libraries and their Application to Drug Discovery" see the whole dcoument; in particular pages 8157-8160	1-22
	X	see page 8158; figure 29 ---	23,24
7	P,X	TETRAHEDRON LETTERS, vol. 37, no. 7, 12 February 1996, OXFORD GB, pages 1003-1006, XP002025142 A.L. MARZINZIK ET AL.: "Solid Support Synthesis of Highly Functionalized Pyrazoles and Isoxazoles; Scaffolds for Molecular Diversity" see the whole document ---	1-24
8	P,X	SYNLETT, no. 7, July 1996, STUTTGART DE, pages 667-668, XP002025143 L.F. TIETZE ET AL.: "A General and Expedient Method for the Solid-Phase Synthesis of Structurally Diverse 1-Phenylpyrazolone Derivatives." see the whole document -----	23,24

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